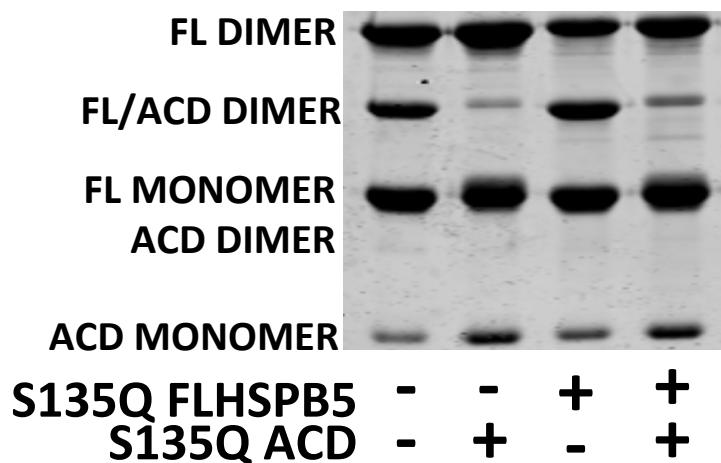
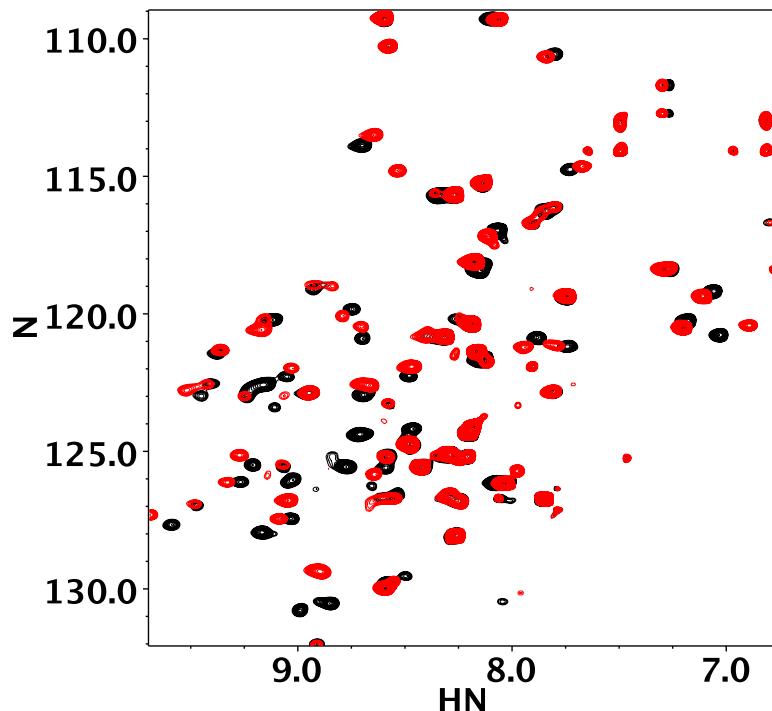


Protein or Protein Mixture	Elution Volume	Apparent Molecular Weight
HSPB5-ACD only	17.7 ml	~30kDa
HSPB6	17.3 ml	~40kDa
ΔN-HSPB6	17.4 ml	~40kDa
S135Q-HSPB5	13.1 ml	~650kDa
HSPB6/S135Q-HSPB5 MIXTURE (Figure 6)	14.1 ml	~330kDa
HSPB6/HSPB5 MIXTURE (Figure 7)	14.4 ml	~270kDa
ΔN HSPB6/HSPB5 MIXTURE (Figure 7)	14.0 ml	~350kDa

S1. Molecular weights versus elution volumes for aSEC: A  $\alpha$ lactalbumin (16kDa), B Yeast Alcohol Dehydrogenase (141kDa), C wheatHSP16.9 (200kDa), D WT-HSPB5 (~480kDa) and E H104K HSPB5 (~810kDa). Average molecular weights for HSPB5 and H104K HSPB5 oligomers are based on reported SEC-MALS values (35). Observed elution volumes and calculated apparent molecular weights for relevant proteins are presented below the plot. Apparent molecular weights calculated based on a linear fit of the  $\log(MW)$  versus elution volume are given.



S2. Non-reducing SDS-PAGE of E117C HSPB5-oligomers and E117C HSPB5-ACDs mixed under reducing conditions, followed by dialysis into oxidizing conditions. Exchange at the ACD-ACD interface is observed by the formation of a mixed FL/ACD locked dimer. Lane 1, the E117C HSPB5-oligomer and E117C-ACD mixture; Lane 2, mixing when the ACD additionally carries the S135Q mutation; Lane 3, mixing when the HSPB5 oligomer additionally carries the S135Q mutation; Lane 4, mixing when both oligomer and ACD additionally carry the S135Q mutation.



S3.  $^{15}\text{N}$ -HSQC Spectrum of  $\Delta\text{N}$ -HSPB6 ( $500\mu\text{M}$ ) in the absence (black) and presence of a peptide (6-fold excess) mimicking a region of the CTR of HSPB5 (Red). Spectra were collected at  $22^\circ\text{C}$ .

HSPB1	---RWRVSLDVNHFAPDELTVKTKDGVVEITGKHEERQDE <i>HGYISRCFTRKYT</i> ---	LPPG	147
HSPB8	---PWKVCVNHSFKPEELMVKTKDGYVEVSGKHEEKQQEGGIVSKNFTKKIO---	LPAE	148
HSPB4	---KFVIFLDVKHFSPEDLTVKVQDDFVEIHGKHNERQDD <i>HGYISREFHRRYR</i> ---	LPSN	123
HSPB5	---RFSVNLDVKHFSPEELKVKVLGDVIEVHGKHEERQDE <i>HGFISREFHRKYR</i> ---	IPAD	127
HSPB6	---HFSVLLDVKHFSPEEI <b>A</b> VKVVGEHVEVHARHEERPDE <i>HGFVAREFHRRYR</i> ---	LPPG	126
HSPB2	---KFQAFLDVSHFTPDEVTVRTVDNLLEVSARHPQRQLDRHGFVSRFCRTYV---	LPAD	126
HSPB3	---HFQI <del>LL</del> DVVQFLPEDII <i>IOTFEGWLLIKAQHGTRMDE</i> <b>HGFISRSFTROYK</b> ---	LPDG	123
HSPB7	---AYEFAVDVDRDFSPEDI <i>I</i> VTTSNHIEVR ---AEKLAADGTVMNTFAHKC---	LPED	131
HSPB9	---GFQM <b>KLDAHGFAPEELVVQVDGQWLMVTGQQQLDVRDPERVSYRMSQKVHRKMLPSN</b>		109
ODF1	CSSNILGSVNVCVGFE <b>DQVKVRVKDGKVCVSAERENRYDCLGSKKYSYMNICKE</b> FSLPPC		180

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HSPB1	VDPTQ <b>VSSSL</b> SPEGTLTVEAPMPKLATQSNE <i>ITIPVTFESRAQLGGPEAAKSDETAAK</i> --		205
HSPB8	VDPVTVFASLSPEGLLIIEAPQVPPYSTFGES---SFNNELPQDSQEVTC-----		196
HSPB4	VDQ <b>SALCS</b> LSADGMLTFCGPKIQTGLDATHAERAIPVSREEKP--TSAPSS-----		173
HSPB5	VDPLT <b>ITSSLSS</b> SDGVLTVNGPRKQ---VSGPERTIPITREEKPAVTAAPKK-----		175
HSPB6	VDPAAV <b>TSALS</b> PEGVLSI-----QAAPASAQAPP--PAAAK-----		160
HSPB2	VDPW <b>RVRAALSH</b> DGILNLEAPRGGRHLDTEVNEYVYISLLPAPPDPEEEEEEAAIVEP--		182
HSPB3	VEIKD <b>LSAVL</b> CHDGILVVEVKD-----PVGTK-----		150
HSPB7	VDPTS <b>VTSAL</b> REDGSLTIRARR-----HPHTEHVQQTFRTE <i>IKI</i> -----		170
HSPB9	LSPTAMTCCLTPSGQLWVRGQCVALALPEAQ <b>TGSPRLG</b> SLGSKASNLTR-----		159
ODF1	VDEKD <b>VTY</b> SYGLGSCVKIESPCYPCTSPCSPCNPCSPCNPCSPYDPCNP <i>CYPCG</i>		240

\*

S4. Sequence alignment of the ACD regions of human sHSPs. The  $\beta$ 4/8 groove involved in CTR binding is highlighted in cyan. The  $\beta$ 6+7 strand, which defines the ACD-ACD dimer interface is highlighted in green. CTR IxI motifs are italicized. Asterisks mark positions E117 and S135 in HSPB5.